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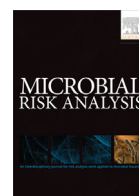
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# Quantitative microbial risk assessment of *Salmonella* in dry fermented sausage (salami) in Southern Brazil



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## ABSTRACT

Dry fermented sausage (salami) is a very popular ready-to-eat product in Southern Brazil, of which the raw materials can be contaminated with pathogens such as *Salmonella*. This product can put consumers at risk if a failure occurs during the manufacturing process. To investigate this risk, a quantitative microbiological risk assessment was performed. The objective was to assess the impact of *Salmonella* inactivation during the process of fermenting and drying and the distribution of the bacteria in minced pork used in Italian-Style salami on the consumer health risk, using a modular process risk model (MPRM) approach. A total of 405 scenarios were tested combining five scenarios for sausage fermentation, three maturation times (12, 15, and 24 days), nine scenarios for prevalence and concentrations of *Salmonella* on pork carcasses, and three scenarios for clustering of cells (homogeneous and heterogeneous). In general, it was observed that the mean exposure to *Salmonella* due to ingestion of a portion of contaminated salami was very low; “zero risks” (with no cases of salmonellosis among 100,000 consumed portions of salami) were found in 65% of the scenarios (265/405) assessed and low risks were found in the other 35% of the scenarios (140/405). Low risks were observed in all scenarios that included 24 days of maturation (0 to  $9.8 \times 10^{-9}$ ;  $n = 135$  scenarios) or  $\geq 2.2$  log reduction at any stage of the process (0 to  $3 \times 10^{-9}$ ;  $n = 189$  scenarios). According to the model, 134 of the 135 scenarios presenting log reduction greater than 3.3 during maturation reduced the mean risk to zero. The most important variables, increasing the risk, were lack of fermentation, short maturation period (12 days), and high concentration of *Salmonella* on the carcass. On the contrary, a negative association (indicating a decreased risk) was observed when 24 days of maturation is applied and/or with good fermentation process. If a realistic heterogeneous distribution of bacteria over the sausages is assumed instead of homogeneous distribution, the estimated risk is larger. Although in general the mean risks found here were low, selling dry fermented sausage before complete maturation of the product and failure in fermentation can pose a risk to the consumers from the studied region. It was found that a maturation period of 24 days can be considered safe, even in a situation with high initial levels of contamination.

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## 1. Introduction

Salmonellosis associated with pork consumption is commonly reported worldwide (Arguello et al., 2013), mainly in regions where pork meat is an important part of the human diet. *Salmonella* is the main pathogen associated with foodborne disease reported in Brazil (Gomes et al., 2013), and the serovar Typhimurium, the one that is most frequently found in swine finishing herds and

slaughter facilities in Southern Brazil (Kich et al., 2011), is also commonly recovered from foodborne outbreaks in the same region (Santos et al., 2013).

In Brazil, pork is the third most consumed meat type, and dry fermented sausages are very popular among Brazilians of Italian and German ancestry. In a study about food consumption habits in Southern Brazil, Oliveira and Betiol (2011) reported that 85% of the people indicated to eat dry sausages, of which 50% eat it regularly. Italian dry sausage (salami) is a ready-to-eat food product made of pork meat, and its quality and safety depends on the raw materials and manufacturing process. Studies in Southern Brazil demonstrated a high prevalence of *Salmonella* in swine population (Schwarz et al., 2010) and in 93.9% of chopped pork used to manufacture sausages (Castagna et al., 2004).

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The principal process steps to reduce the presence of pathogens in dry sausages are fermentation and drying, the oldest methods of food preservation and preparation (Mehta et al., 2012). As a result of these processes, there are multiple antimicrobial barriers in fermented sausage, such as low pH and  $a_w$  (water activity); curing agents as nitrite salts and salt are hurdles for pathogens as well (Hutkins, 2006). Control of fermentation may have the greatest impact on assuring the safety of the final product (Hutkins, 2006). On the other hand, spontaneous fermentation processes have a high risk of failure (Holzapfel, 2002), and these are typically used by small manufacturers in the South of Brazil (Dalla Santa et al., 2012).

Drying times for fermented sausage like salami must ensure a final  $a_w$  between 0.85 and 0.91, and the Brazilian legislation specifies a maximum  $a_w$  of 0.90 (Brasil, 2000). Nonetheless, a study performed with samples of salami known as “colonial sausage”, which is a very popular dry fermented sausage produced by small scale food manufacturers in Southern Brazil, reported that 65% of them had an  $a_w$  larger than 0.92. This means that the product may get into the retail before finalization of the whole maturation process.

Considering that salami is a very popular ready-to-eat product and that the raw materials can be contaminated with pathogens such as *Salmonella*, this product can put consumers at risk if a failure occurs during the manufacturing process. Conversely, there are few reported cases of salmonellosis due to consumption of salami in the region. To investigate the public health risks of salmonellosis related to the consumption of salami quantitative microbial risk assessment (QMRA) is a useful approach and a good alternative when surveillance data are sparse (Pouillot et al., 2012). In this study we performed a QMRA using the modular process risk model (MPRM) approach (Nauta, 2008), as previously used in food chain risk assessments of pork (e.g. Møller et al., 2015; Swart et al., 2016). The objectives of the QMRA were to assess the impact of both *Salmonella* inactivation during the process of fermenting and drying and its distribution in the pork minced meat used in Italian-Style salami on the mean risk for consumers.

## 2. Material and methods

### 2.1. Italian-Style salami and units used in the model

Italian-Style salami is a dry fermented sausage in which the basic ingredients are pig meat, fat (lard), salt, curing agents and flavorings. The amount of pig meat varies approximately from 60% to 80% of the total amount of ingredients of the salami formulation, where most meat used is cut from the shoulder of the pig (expert information from meat processors, data not shown). The maturation process considered here is the fermentation followed by drying phases, in which the difference between them is basically the temperature and relative humidity applied. The fermentation parameters vary depending on the desired product and can take as long as two to five days (Spricigo and Pianovsky, 2005). In general, drying times depend on the product specification, and for dry fermented sausages such as salami the drying time should be long enough to lose about 35% of water and to reach an  $a_w$  between 0.85 and 0.91 (Hutkins, 2006).

For the QMRA, the units of the products applied in the model have to be defined (Nauta, 2008). The units used in the QMRA were (Kelley et al., 1973; Oliveira, 2011; Peet, 2013; expert information from meat processors, data not shown): 1) pig carcass and shoulder surface area of 16,500 cm<sup>2</sup> and 2300 cm<sup>2</sup>, respectively; 2) weight of deboned shoulder of 5 kg; 3) weight of mixed meat batch/day of 1000 kg; 4) unit of salami of 0.25 Kg; and 5) servings of 20 g. It was assumed in this model that the salami contains 80% swine meat, and therefore, for a 1000 kg batch, 80 carcasses

per day are necessary to obtain 800 kg of pig meat, considering that carcasses contain on average 5 kg of meat in each shoulder.

### 2.2. Prevalence and concentration of *Salmonella* in pig carcasses

Prevalence data of *Salmonella* in pork carcasses were obtained from studies performed in slaughterhouses in southern Brazil (Corbellini et al., 2016; da Silva et al., 2012; Pissetti et al., 2012; Kich et al., 2011). *Salmonella enterica* serovar Typhimurium was one of the most common serovar isolated in these studies (da Silva et al., 2012; Pissetti et al., 2012; Kich et al., 2011). The percentage of *Salmonella*-positive carcasses on each day of sampling were used, and these data were fit to a Beta ( $\alpha, \beta$ ) distribution, where the parameters  $\alpha$  and  $\beta$  were obtaining using the MATCH Uncertainty Elicitation Tool (Morris et al., 2014).

Five prevalence scenarios were used to describe the between day variation in carcass prevalence: 1) scenario slaughterhouse A (low average prevalence); 2) scenario slaughterhouse B (high average prevalence); 3) scenario slaughterhouse C (low/medium average prevalence), all of them obtained from da Silva et al. (2012); 4) scenario “all” which included data from all the slaughterhouses mentioned above, as well as data from largest slaughterhouses in the region (Corbellini et al., 2016); and 5) scenario “all reduced” in which the 5% highest prevalence values were excluded (i.e. prevalences greater than 40%).

*Salmonella* concentrations (log cfu/cm<sup>2</sup>) on carcass surface were obtained from da Silva et al. (2012). In this study, carcasses sampled from slaughterhouses A, B, and C were screened for *Salmonella* and enumerated by the most probably number (MPN) method. *Salmonella* contamination data were fitted to a lognormal distribution separately for slaughterhouse A and C by using a Bayesian model that uses the number of positive tubes at each dilution in an MPN analysis to estimate the parameters of the concentration distribution (Corbellini et al., 2015). The concentration of *Salmonella* from the carcasses collected in slaughterhouse B was very high, and the Bayesian model did not converge, therefore, data were fit to log normal distribution using the model described by Pouillot et al. (2013).

The obtained distributions for the concentration ( $N$ ) of *Salmonella* (log cfu/cm<sup>2</sup>) in the three slaughterhouses were A: Normal (−4.64, 0.51); B: Normal (−2.62, 1.00); and C: Normal (−3.6, 0.91). These distributions describe the variability in concentrations between contaminated carcasses in each slaughterhouse.

### 2.3. Food pathway and the modular process risk model (MPRM) framework

A description of the food pathway from swine prechill carcasses up to the finished product was made, followed by the definition of the MPRM structure. After chilling, the carcasses proceed to the cutting plant, where cuts of pork shoulders are removed to produce salami, following the process of chopping and grinding, mixing the ingredients with the grinded meat, stuffing and fermentation/drying (Table 1). In this study, the QMRA model was implemented in the software @Risk, version 6.0 (Palisade, Newfield). Monte Carlo simulations were performed with 100,000 iterations. Each iteration simulates a batch and a slaughter day, with 80 carcasses slaughtered per day. Each day, the shoulders of these carcasses are collected in one batch of chopped meat, ingredients are added, and sausages are produced from this batch, as explained below. A simulation therefore represents 100.000 batches/slaughter days, which showed to be sufficient to assess mean values and get a good impression of the variability.

For each iteration, the carcass prevalence ( $P_c$ ) is sampled from the appropriate Beta distribution given in Table 1. The number of

**Table 1**

Description of the model variables and parameters, food pathway and the modular process risk model structure (MPRM). Each iteration of the model corresponds to 20 sausages/day sampled from a batch containing 1000 kg that, in turn, is composed of 80 pig carcasses.

MPRM/Food pathway	Variable	Scenarios and parameters
Starting point: Prechill carcass	Carcass prevalence ( $P_C$ ) (daily variation)	$A \sim \text{Beta}(0.13, 5.54)$ $B \sim \text{Beta}(0.41, 1.05)$ $C \sim \text{Beta}(0.29, 2.95)$ "all" $\sim \text{Beta}(0.41, 3.08)$ "all reduced" $\sim \text{Beta}(0.47, 10.78)$ $\sim \text{Binomial}(80, P_C)$
–	Number of contaminated carcasses, $n_C$	
–	<i>Salmonella</i> concentration, log cfu/cm <sup>2</sup> ( $N$ )	$A$ (very low) $\sim \text{Normal}(-4.56, 0.51)$ $B$ (high) $\sim \text{Normal}(-2.62, 1)$ $C$ (low) $\sim \text{Normal}(-3.6, 0.91)$ Round ( $16,500 \times 10^N$ ) for contaminated carcass, otherwise 0.
Partitioning /cutting shoulder	$N'_{u \text{ shoulder}, i}$	$\sim \text{Binomial}(N_{\text{carc}}, 2^* s_{u \text{ shoulder}}/S_{\text{carcass}})$ $s_{u \text{ shoulder}}$ is the surface of the shoulder (2500 cm <sup>2</sup> ), and $S_{\text{carcass}}$ is the carcass surface (16,500 cm <sup>2</sup> ).
Mixing/chopped meat and ingredients	$N'_{\text{batch}}$	$\sum_{80} N'_{u \text{ shoulder}}$
Partitioning/stuffing	$N'_{u \text{ sausage}}$	Homogeneous $\sim \text{Binomial}(N'_{\text{batch}}, s_{u \text{ sausage}}/S_{\text{batch}})$ Heterogeneous $\sim \text{Binomial}(N'_{\text{batch}}, \text{Beta}(b, b(n-1)))$ $s_{u \text{ sausage}}$ is the unit of a sausage (0.25 kg) and $S_{\text{batch}}$ is the size of batch (1000 kg); $n$ is total number of sausage from a batch (i.e., 4000), and $b$ is the 'clustering' parameter.
Survival/fermentation and drying	$\log(N'_{u \text{ Maturation}})$	$\log(N'_{u \text{ Maturation}}) - g(\cdot)$ Scenarios for fermentation please see Table 2. Where $g(\cdot)$ is given by: $g(t) = \sum_{\forall t} \frac{\Delta t}{D(t, pH, aw)}$ $D$ -value is the decimal reduction time in a given temperature, pH and aw in a time interval $\Delta t$
Servings/exposure	$N'_{\text{portion}}$ (dose 'd' in a portion of salami)	$\sim \text{Poisson}(N'_{u \text{ Maturation}} * s_{u \text{ portion}}/S_{u \text{ Maturation}})$ $N'_{u \text{ Maturation}}$ is the number of <i>Salmonella</i> in a sausage $i$ after maturation (fermentation and drying), and $s_{u \text{ portion}}$ and $S_{u \text{ Maturation}}$ is the portion of 20 g and the unit of salami of 250 g, respectively.
Risk characterization	Dose-response	$P_{\text{ill}} = 1 - (1 + \frac{N'_{\text{portion}}}{2885})^{-0.3126}$

contaminated carcasses among the 80 carcasses slaughtered ( $n_C$ ), is then sampled from a Binomial ( $80, P_C$ ) distribution. For each of these  $n_C$  contaminated carcasses, the number of *Salmonella* per carcass ( $N_{\text{carc}}$ ) is obtained by rounding off the value for  $10^N \times 16,500$  (i.e., carcass surface area), where  $N$  is sampled from the appropriate distribution for the concentration (log cfu/cm<sup>2</sup>), given in Section 2.2. and table 1. For uncontaminated carcasses,  $N_{\text{carc}} = 0$ .

The modules of the MPRM cover processing steps and describe the changes in the number of pathogen per unit of food product and the prevalence of units of food product contaminated (Nauta, 2008). The basic processes used along the food pathway were (Table 1): 1) partitioning (cutting room, removing shoulders from the carcasses); 2) mixing (grinding chop meat and mixing ingredients); 3) partitioning (stuffing salami); and 4) survival (during the fermenting and drying processes).

Partitioning occurs when a large unit is split up into several small units and is modelled as described by Nauta (2005). With this method we can estimate the number of small units without *Salmonella* cells and the distribution of cells in the contaminated ones after splitting. In this QMRA, partitioning models were used in two steps in the food pathway as described above. First, the carcass, the large unit containing  $N_{\text{carc}}$  *Salmonella* cells (cfu per carcass), is split up in the cutting room, and the two small units, i.e. the shoulders, contain  $N'_{u \text{ shoulder}}$  *Salmonella* cells. Considering that the shoulder is a small unit, and the carcass is the large unit containing  $N_{\text{carc}}$  *Salmonella* cells homogeneously distributed over the carcass,  $N'_{u \text{ shoulder}}$  is given as a sample from a Binomial distribution (indicated by  $\sim$ ):

$$N'_{u \text{ shoulder}} \sim \text{Binomial}(N_{\text{carc}}, 2^* s_{u \text{ shoulder}}/S_{\text{carcass}}) \quad (1)$$

where  $s_{u \text{ shoulder}}$  is the surface of each of the two shoulders (2300 cm<sup>2</sup>), and  $S_{\text{carcass}}$  is the carcass surface (16,500 cm<sup>2</sup>). In the QMRA model, Eq. (1) was applied for each of the 80 carcasses that compose a daily batch. If  $N_{\text{carc}} = 0$ , so is  $N'_{u \text{ shoulder}}$ .

Meat from the shoulders will be collected and joined into the daily batch. With this mixing process, the number of *Salmonella*

cells in the batch ( $N'_{\text{batch}}$ ) is the sum of the number of *Salmonella* in the 80 pairs of shoulders ( $N'_{u \text{ shoulder}}$ ) from the carcasses that compose a batch (Nauta, 2005).

For the other partitioning process, the same method as for cutting up the carcass was used to estimate the number of *Salmonella* cells after stuffing salami. In the model, 1000 kg of mixed ingredients, the large unit  $S_{\text{batch}}$ , produces 4000 sausages of 0.25 kg each, i.e., the small units  $s_{u \text{ sausage}}$ . Therefore, considering that the cells are homogeneously distributed in the mixed meat, the number of cfu in each sausage  $i$   $N'_{u \text{ sausage}, i}$  is obtained by:

$$N'_{u \text{ sausage}, i} \sim \text{Binomial}(N'_{\text{batch}}, s_{u \text{ sausage}}/S_{\text{batch}}) \quad (2)$$

where  $s_{u \text{ sausage}}$  is the unit of a sausage (0.25 kg) and  $S_{\text{batch}}$  is the size of batch (1000 kg).

The partitioning model described in (2) does not include the dependence between the number  $N'_{u \text{ sausage}, i}$  in the sausages. The dependence needs to be incorporated because cells that are allocated to one small unit cannot be allocated to another (Nauta, 2005). Therefore, the Multinomial distribution was used as an alternative to the Binomial and implemented in @Risk by recursive sampling from the Binomial distribution, as described in Appendix A in Nauta (2005). A total of 20 sausages  $i$  were sampled from the Binomial distribution. Consequently, each iteration of the model corresponds to 20 sausages/day sampled from a batch containing 1000 kg that, in turn, is composed of 80 pig carcasses. These 20 sausages per batch were found to be sufficient to capture the variability between the (approximately) 1000 kg of sausages produced from one batch, which is considerably smaller than the variability between batches.

Heterogeneous (i.e., clustered) distribution of *Salmonella* cells can occur due to cell clustering or incomplete mixing of contaminated chopped pork meat (Jongenburger et al., 2012). Therefore, as an alternative method to describe the second partitioning process, a betabinomial distribution was applied to test different scenarios of *Salmonella* cells distribution in units of sausages from

**Table 2**Five scenarios describing good and low quality fermentation process and different values of pH and  $a_w$  of the raw sausage at the beginning of the process.

Scenarios (label)	General characteristics	Sources
G1	Good fermentation <sup>1</sup> and low initial values of both $a_w$ (0.96) and pH (5.6). This represents the best scenario (baseline) for sausage production characterized by good raw material pH and $a_w$ values and optimal fermentation.	Degenhardt and Sant'anna (2007a); Cirolini et al. (2010).
G2	Good fermentation and moderate initial values of $a_w$ (0.98) and high pH (6).	Cirolini et al. (2010), Stahnke and Tjener (2007).
G3	Good fermentation and high initial values of $a_w$ (0.99) and pH (6.0).	Cirolini et al. (2010), Comi et al. (2005), Toldrá (2015)
B1	Sub-optimal fermentation <sup>2</sup> and moderate initial values of $a_w$ (0.98) and high pH (6.1).	Campagnol et al. (2007), Toldrá (2015).
B2	Failure in fermentation <sup>2</sup> and moderate initial value of $a_w$ (0.98) and high pH (6.2). This represents the worst-case scenario for sausage production characterized by low quality raw material, i.e., very high pH and failure in fermentation.	Cirolini et al. (2010), Sprigco and Pianovsky (2005), Toldrá (2015).

<sup>1</sup> pH drops to around 5 within 72 h.<sup>2</sup> In sub-optimal fermentation process and failure in fermentation scenarios, the pH dropped to around 5.7 and 6.1 within 72 h, respectively.

homogeneity (random, formula 2) to heterogeneous by decreasing the 'clustering' parameter ( $b > 0$ ) of the distribution, that is (Nauta, 2005):

$$N'_{u \text{ sausage}, i} \sim \text{Binomial}[(N'_{\text{batch}}, \text{Beta}(b, b(n-1)))] \quad (3)$$

where  $N'_{\text{batch}}$  is the number of *Salmonella* cells in a batch,  $n$  is total number of sausages from a batch (i.e., 4000), and  $b$  is the 'clustering' parameter; if  $b$  approximates zero, there is maximum clustering, if  $b$  approximates infinity the distribution is homogeneous. Scenarios using  $b$  values of 0.5 and 1 were tested.

#### 2.4. Fermentation and drying process (survival model)

During maturation, the fermentation and drying conditions vary depending on the product quality and the culture (Degenhardt and Sant'anna, 2007a; Hutkins, 2006). The fermentation and drying conditions applied here were 72 h at 24 °C, 72 hours at 20 °C and 18 days of drying at 14 °C, resulting in 24 days of maturation in total. These parameters were obtained from several meat processors in Southern Brazil for a sausage with an average size around 50 mm (data not shown).

The *Salmonella* concentration in a 250 g sausage after this process (cfu) can be obtained from a general formula for modeling inactivation (Nauta, 2008):

$$(\log(N'_{u \text{ maturation}})) = \log(N'_{u \text{ sausage}}) - g(\cdot) \quad (4)$$

where  $g(\cdot)$  is an inactivation function and log is the 10-based log. Here, the Bigelow model was used for modeling the survival process; this is a linear function in time ( $t$ ) with a shape  $g(t) = t/D$ , where  $D$ -value is the decimal reduction time in a given temperature, pH and  $a_w$  in a time interval  $\Delta t$ . Under dynamic conditions, with changing temperature  $T_t$ , acidity  $pH_t$  and water activity  $a_{wt}$ ,  $g(t)$  is calculated as (Nauta, 2001):

$$g(t) = \sum_{\forall t} \frac{\Delta t}{D_{(T_t, pH_t, a_{wt})}} \quad (5)$$

$D$ -values were obtained through the non-thermal survival model of the ComBase software (USDA, 2016) using the parameters of pH,  $a_w$  and temperature. The  $\log(N_{\text{out}})$  along the process was evaluated over time intervals  $\Delta t$  as follow: 24 h, 48 h and then each 72 hs pH and  $a_w$  values along these intervals were obtained from the literature. Based on the data acquired, five scenarios describing good and low quality fermentation processes and different values of pH and  $a_w$  of the raw sausage at the beginning of the process were described (Table 2). Values were obtained from the referenced paper's tables or, if demonstrated with graphics, using the software GetData (GetData Graph Digitizer version

2.26.0.20) from several studies made in the region (Campagnol et al., 2007; Cirolini et al., 2010; Degenhardt and Sant'anna, 2007b; Sprigco and Pianovsky, 2005). In general, the pH decreases linearly in the first 72 – 144 h, and subsequently tends to stabilize or increase at the end of drying, while the  $a_w$  decreases gradually during the maturation process (data not shown). Changes in pH and  $a_w$  for the five scenarios are shown in Fig. 1.

In some combinations that included high pH and  $a_w$  in the raw materials, growth of *Salmonella* can theoretically occur at the beginning of the maturation process, i.e., during the fermentation. In this case -  $g(\cdot)$  in Eq. (4) is replaced by an (increasing, positive) growth function; this growth rate value (log/hour) was obtained in ComBase (USDA, 2016).

#### 2.5. Exposure assessment and risk characterization

The number of *Salmonella* (cfu) in a portion of 20 g of salami in a meal was obtained by application of a Poisson distribution:

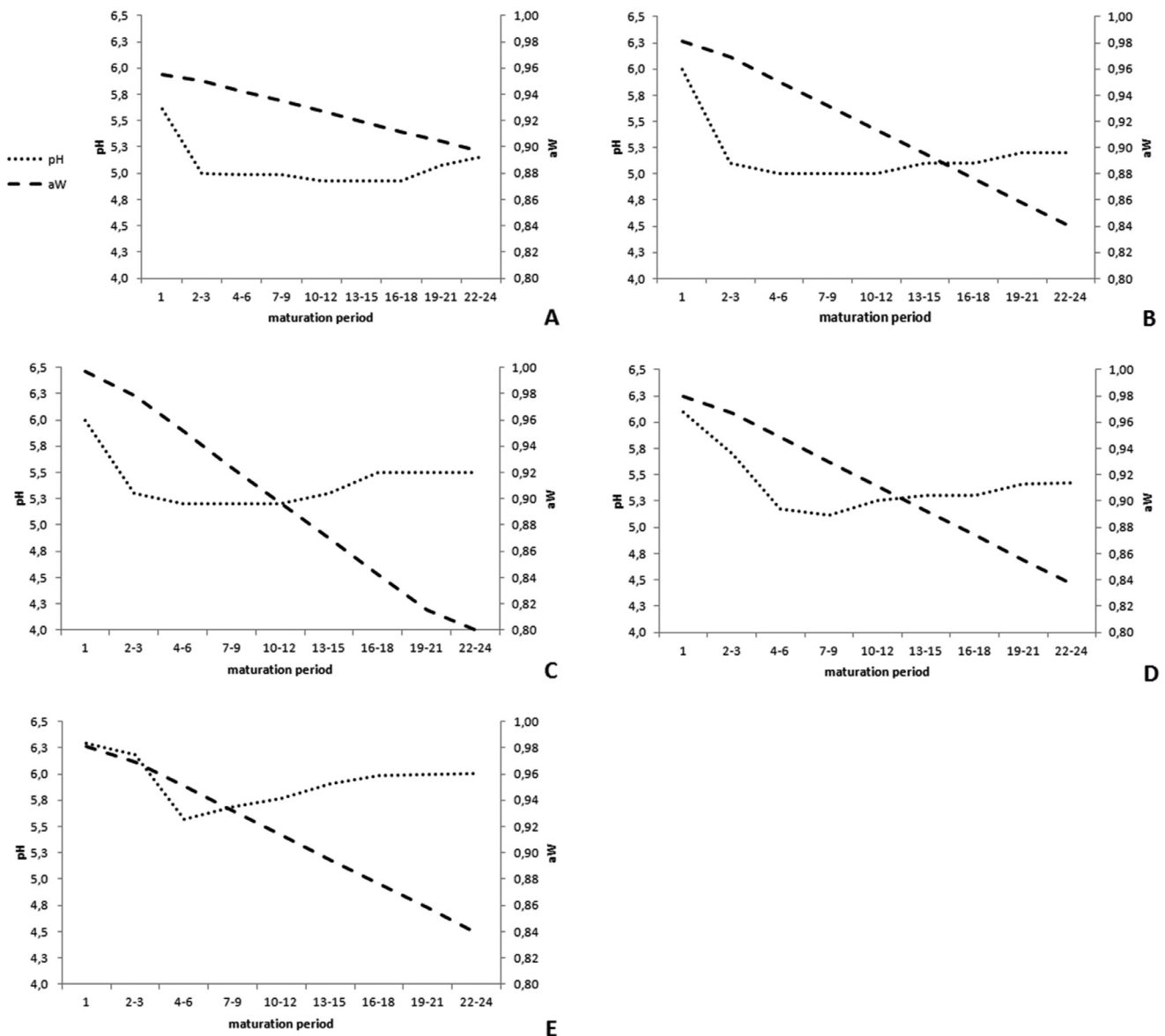
$$N'_{\text{portion}} \sim \text{Poisson}(N'_{u \text{ maturation}} * (S_{u \text{ portion}}/S_{u \text{ sausage}})) \quad (6)$$

where  $N'_{u \text{ maturation}}$  is the simulated number of *Salmonella* in a sausage  $i$  after fermentation and drying, and  $S_{u \text{ portion}}$  and  $S_{u \text{ sausage}}$  is the portion of 20 g and the unit of salami of 250 g, respectively. Assuming a homogeneous distribution within the sausage, the Poisson distribution is used to account for partitioning and to get a discrete dose. For the exposure assessment, 20 values for  $N'_{\text{portion}}$  from 20 sausages per batch were obtained. These were used as doses in the dose response relation to estimate de probability of illness per serving ( $P_{\text{ill}}$ ). The Beta Poisson model published by FAO/WHO (2002) with  $\alpha = 0.3126$  and  $\beta = 2885$  was used for this purpose, and the mean probability of illness was calculated for 20 sausages from 100.000 batches. These parameters are suitable for general population and all the serotypes could be adequately described using a single beta-Poisson dose-response curve (FAO/WHO, 2002):

$$P_{\text{ill}} = 1 - \left(1 + \frac{N'_{\text{portion}}}{2885}\right)^{-0.3126} \quad (7)$$

The population greater than 10 year of age of the State of Rio Grande do Sul, southern Brazil, is 7.929.511 inhabitants (IBGE, 2000). The average consumption of salami per person/year is 398 g (IBGE, 2010); considering that 85% of the population eat salami (Oliveira and Betiol, 2011), the number of servings per year is approximately 135.594.638. The number of cases was estimated by multiplying this number of servings with the mean probability of illness ( $P_{\text{ill}}$ ).





**Fig. 1.** Scenarios used in the models to represent the parameters of water activity ( $a_w$ ) and pH during the maturation process (fermentation and drying) of Italian-style salami. Five combinations of survival models that reflect the fermentation process (first 72 h) were tested. (A) Good fermentation and low initial values of both  $a_w$  (0.96) and pH (5.6) (model label G1). (B) Good fermentation and moderate initial values of  $a_w$  (0.98) and high pH (6) (model label G2). (C) Good fermentation and high initial values of  $a_w$  (0.99) and pH (6.0) (model label G3). (D) Sub-optimal fermentation and moderate initial values of  $a_w$  (0.98) and high pH (6.1) (model label B1). (E) Failure in fermentation and moderate initial value of  $a_w$  (0.98) and high pH (6.2) (model label B2).

## 2.6. Variability and main scenarios analysis

Variability is included in the model as explained in Section 2.3, and the uncertainties were attained through the construction of scenarios as explained below. Therefore, each iteration represents a batch and a day, so different iterations represent variation between days and this includes variation in carcass prevalence per day. Thus, the number of contaminated carcasses used for a batch per day is modelled, and the model provides the consequential per-day variation of sausage prevalence in each scenario evaluated.

To study the uncertainty attending the model and to explore the effects of interventions, several scenarios ( $n = 405$ , Table 3) were assessed through different combinations of model parameter values, addressing failure during the fermentation/drying process that can pose a risk to the underlying population, uncertainty about the parameter values used, and scenarios that describe different options for processing:

**Fermentation process (five scenarios):** Five combinations of survival models that reflect the fermentation process were tested (G1, G2, G3, B1, and B2 Table 2, Fig. 1). Different process options were assessed to verify what happens if the processes of fermentation/drying are suboptimal and/or if low quality raw materials are used.

**Maturation times (three scenarios):** these scenarios represent three situations (12, 15 and 24 days) that have been observed in Southern Brazil (Degenhardt and Sant'anna, 2007b; Dalla Santa et al., 2012), where the product is sold before maturation. These scenarios are evaluated to study the risk of selling the product before the maturation is finalized, which, for medium-fermented salami process, is around 3–4 weeks.

**Carcass prevalence (five scenarios):** three scenarios containing data from the three slaughterhouses (A, B, and C) and one that included data from several studies ("all") were tested (see Section 2.2). These scenarios express the impact of the prevalence. In one

Description of the 405 scenarios with all the combination of parameters used in the QMRA. The dark grey represents scenarios that resulted in at least one case of *Salmonella* (n = 72) if these scenarios would be applied to all servings (135,594,638) and light gray represents scenarios (n = 68) that resulted in very low risk (less than one expected case of *Salmonella*). The other scenarios resulted in “zero risks” with no cases in 100,000 model iterations (n = 265). Combination of fermentation scenarios (G1 to B2), *Salmonella* prevalence in pork carcasses (A, B, C, “all”, and “all reduced”), *Salmonella* concentrations (A, B, C), maturation times (12, 15, and 24 days), and for clustering of cells (homogeneous “ho” and heterogeneous “h”; the latter has distinct clustering parameters ‘b’, that is 0.5 for “h1” and 1 for “h2”) were assessed.

[illegible]

<sup>1</sup> Concentration: A: Normal (−4.64, 0.51); B: Normal (−2.62, 1); and C: Normal (−3.6, 0.91); <sup>2</sup> Fermentation: Five process options from optimal to suboptimal fermentation condition and/or low quality raw materials; G1 represents the best scenario and B2 the worst scenario; <sup>3</sup> Maturation times in days; <sup>4</sup> Reference scenario for sensitivity analysis.

scenario, named “all reduced”, an intervention was tested in which the carcass prevalences were reduced.

**Concentration (three scenarios):** these reflect the uncertainty about the concentration of *Salmonella* in the pork carcass. The distributions of concentrations in three slaughterhouses (A, B, and C) were compared, as explained in Section 2.2 (da Silva et al., 2012). Per slaughterhouse, these distributions were combined with the day variation in prevalence using the respective prevalence distribution obtained in each slaughterhouse (i.e., variation in carcass prevalence per day observed in A along with concentration obtained in A). Additionally, for the prevalence scenarios “all” and “all reduced”, in which a large number of slaughterhouses were included in a microbial survey, both were used in combination with the three concentration distributions from slaughterhouses A, B and C (Table 3). This yields a total of nine scenarios with different initial carcass prevalences and concentrations.

**Partitioning during stuffing of sausages (three scenarios):** Homogeneous distributions and two fixed values of ‘clustering’ parameters for heterogeneous distributions (parameter  $b=0.5$  and 1) were run. These scenarios evaluate uncertainties about the clustering of cells in the dry sausage after stuffing, assuming that heterogeneous distribution of *Salmonella* cells can occur due to incomplete mixing of contaminated chopped pork.

## 2.7. Additional scenarios

First, the uncertainty around the distribution of *Salmonella* cells that is a consequence of the heterogeneous distribution that can occur due to incomplete mixing of contaminated chopped pork meat was analysed. The scenario with good fermentation (G3), 15 days of fermentation/drying process and high prevalence (B) and concentration of *Salmonella* in carcasses (B) was tested from ‘perfect’ homogeneity (random) to heterogeneous varying the ‘clustering’ parameter ( $b > 0$ ) of the distribution. The values tested were (from higher to lower heterogeneous distribution): 0.1, 0.25, 0.5, 0.75, 1, and 10. For illustration, a scenario with lower (10 g) and higher (40 g) serving size of salami was assessed using the same parameters above, assuming ‘perfect’ homogeneity.

Next, cross contamination that can occur in the cutting room is hard to describe and is not incorporated in the model. It is assumed that the number of *Salmonella* present on the surface of the shoulder will end up in the batch containing the mixed ingredients, without considering cross contamination between carcasses during cutting. Since it is difficult to model the amount of bacteria that is transferred from the knife to the meat or from the other pork meat cut to the shoulder, we run an alternative scenario considering a reduced mean number of the concentration of *Salmonella*. Thereafter, it was assumed that a lower number of bacteria than the present in the surface would end in the meat for salami production. The mean of the highest concentration scenario (B) was reduced by 0.25 log, resulting in ( $\sim$  Normal ( $-2.87$ , 1)). To check this, the worst-case values for prevalence (B) and fermentation were tested.

Moreover, fixed log reductions of the entire process (0.5, 1, 2, 2.5, 3, 3.25, 3.5 and 3.75 logs) were applied to the models to assess the mean risks using the worst parameter of prevalence in the following additional scenarios: 1) highest concentration of *Salmonella* and homogeneous distribution; 2) highest concentration of *Salmonella* and heterogeneous distribution ( $b=0.1$ ); and 3) reduced mean of the *Salmonella*’s highest concentration distribution (see above), and homogeneous distribution.

## 2.8. Sensitivity analysis

The numbers of cases obtained from each of the 405 scenarios were log transformed. After that a log relative risk was calculated

for each scenario using the scenario with good fermentation process (G1), 15 days of maturation, heterogeneous distribution of *Salmonella* cells in the batch (‘clustering’ parameter  $b=0.5$ ) and both high prevalence (B) and concentration of *Salmonella* in the carcasses (B) as the reference scenario. This scenario was chosen because it was the only one with a good fermentation process that resulted in an observable risk (larger than 0) after 15 days of maturation.

A regression model using the PROC GLM of the software SAS was made to identify the most influential variable in the model using as response variable (Y) the log relative risk and the explanatory the following categorical variables (X): prevalence (A, B, C, “all”, and “all reduced”), *Salmonella* concentration (A, B, C) fermentation scenarios (G1, G2, G3, B1, B2), cells distributions (homogeneous, heterogeneous), and days of maturation (12, 15, 24). Least square means were calculated for each effects (explanatory variables) through the statement LSMEANS and plotted in a graph.

## 3. Results

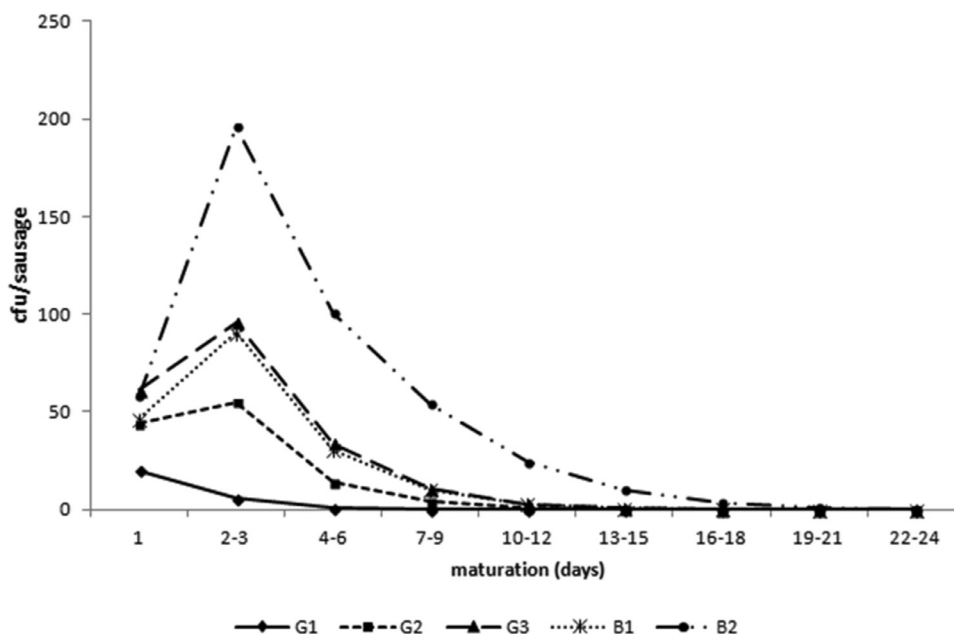
### 3.1. General results and main scenarios analysis

A total of 405 scenarios were tested combining distinct parameters for fermentation (G1, G2, G3, B1 and B2), maturation times (12, 15, and 24 days), *Salmonella* prevalence (A, B, C, “all”, and “all reduced”) and concentration (A, B, and C) and clustering of cells (homogeneous and heterogeneous). Table 3 presents the description of the scenarios with all the combination of parameters and depicts the ones that resulted in risk.

The mean prevalence of *Salmonella* in carcasses  $P_C$  (95% C.I.) for the scenarios A, B, C were 2.4% (0.0%–20.7%), 27.9% (0.01%–92.9%) and 9.0% (0.0%–50.9%), respectively, obtained from Beta distributions describing the per day variation, using slaughterhouse data (da Silva et al., 2012). The mean prevalence of contaminated carcasses when a broad number of studies were included (i.e., scenario “all”) was 11.8% (0.003% – 55.2%), and when a reduced prevalence is tested (“all reduced”) it was 4.2% (0.003% – 20.6%). During the partitioning of the carcass, for scenarios including low concentration of *Salmonella* (A;  $n=135$ ), the probability of a shoulder containing more than one *Salmonella* cell given that the carcass was contaminated was about 10% (between 9.9% and 10.3% among the 135 scenarios). In contrast, for scenarios including high concentration of *Salmonella* (B;  $n=135$ ), the probability of a shoulder containing more than one *Salmonella* cell was about 81% (between 81.3% and 81.6%), whereas it was about 50% in scenario C (between 50.2% and 50.6%).

The number of *Salmonella* cells in one sausage after stuffing was generally low in all the scenarios, and the maturation process reduced the concentration of *Salmonella* even more. In the five scenarios used to reflect different fermentation processes, the *Salmonella* concentration was reduced by 2, 3.5, 3.7, 4.5, and 5.4 log cfu/sausage after 24 days of maturation for G1, G2, G3, B1 and B2 (see Table 2 for definition of these scenarios), respectively. In scenarios where the initial parameters of pH and  $a_w$  were high (G3, B1, and B2), particularly in the ones that simulate fermentation failure (B1, B2), growth of *Salmonella* occurred. Fig. 2 depicts growth and survival of *Salmonella* cells in one sausage representing the 99.9th percentile after 100,000 iterations, in the scenario with the highest parameters values for both carcass prevalence (B) and concentration of *Salmonella* (B) and homogeneous distributions. It shows that even in the tail of the distribution of *Salmonella* concentration in one sausage (99.9th percentile), no cells were found after 24 days of fermentation and drying. According to the model, only when the fermentation failed (scenario B2) two *Salmonella* cells were found at the 100th percentile after 24 days.





**Fig. 2.** Growth and survival of *Salmonella* cells in one sausage (cfu/sausage) during maturation process in a scenario with the highest parameters values for both carcass prevalence (mean of 27.9%) and concentration of *Salmonella* in the pork carcass (Normal  $(-2.62, 1)$ ). Five combinations of survival models that reflect the fermentation process were tested, and according with the legend: (G1) good fermentation and low initial values of both aw (0.96) and pH (5.6); (G2) good fermentation and moderate initial values of aw (0.98) and high pH (6); (G3) good fermentation and high initial values of aw (0.99) and pH (6.0); (B1) sub-optimal fermentation and moderate initial values of aw (0.98) and high pH (6.1); (B2) failure in fermentation and moderate initial value of aw (0.98) and high pH (6.2). As the vast majority of sausages are uncontaminated, the 99.9th percentile of the distribution obtained from a Monte Carlo simulation with 100,000 iteration is shown. The  $\log(N_{out})$  along the process was evaluated over time intervals  $\Delta t$  as follow: 24 h, 48 h and then each 72 h.

In general, “zero risks” (i.e., scenarios that resulted in “zero cases” among 100,000 consumed portions of salami) were found in 65% of the scenarios (265/405) assessed and low risks were found in the remaining 35% of the scenarios (140/405). One or more cases of salmonellosis caused by the intake of *Salmonella* from the contaminated portion of salami were expected in 72 of the 405 (17.8%) scenarios tested, while in 68 the risks were so low that less than one case was expected. Table 4 presents results of the main outputs assessed in the models using five fermentation processes (G1 to B2) with 12, 15, and 24 days of maturation combined with the worst scenario for both carcass prevalence (B) and concentration of *Salmonella* in the carcass (B), and homogeneous distributions. In general, it was observed that the mean exposure to *Salmonella* due to ingestion of a portion of contaminated salami was very low. In the worst-case scenario described in Table 4, which included failure in fermentation (B2) and 12 days of maturation process, the mean probability of illness (risk) found was  $7.7 \times 10^{-6}$ . If this worst-case scenario would be applied to all servings ( $\sim 135,600,000$ ), it would result in 1044 cases.

Low risks were observed in all scenarios that included 24 days of maturation ( $0$  to  $9.8 \times 10^{-9}$ ;  $n=135$  scenarios) or  $\geq 2.2$  log reduction at any stage of the process ( $0$  to  $3 \times 10^{-9}$ ;  $n=189$  scenarios). According to the model, 134 of the 135 scenarios presenting log reduction greater than 3.3 during maturation reduced the mean risk to zero. These scenarios included combinations that involved 15 and 24 days of maturation, and fermentation from good to sub-optimal (G1, G2, G3, and B1) along with any parameter for concentration and/or prevalence. From these 135 scenarios, the one that resulted in a mean risk of  $5.4 \times 10^{-11}$  was the only one with an observable risk among all combinations containing 15 days of maturation and good fermentation (G1) and included both high *Salmonella* carcass prevalence and concentration, and heterogeneous distribution of *Salmonella* cells ( $b=0.5$ ).

### 3.2. Additional scenarios

A more homogeneous distribution of *Salmonella* cells, characterized by higher values of the clustering parameter  $b$ , resulted in a higher prevalence of salami contaminated with *Salmonella* after stuffing, right before maturation (fermentation and drying). However, the opposite was observed after the maturation process, where the prevalence of contaminated salami increased when the distribution after stuffing was more heterogeneous (i.e., lower values of  $b$ ; see Table 5). The risks for distinct serving sizes were  $7.2 \times 10^{-9}$ ,  $1.5 \times 10^{-8}$  and  $2.5 \times 10^{-8}$  for 10, 20, and 40 g respectively.

Using fixed values for log reductions for the maturation process along with the parameter of highest *Salmonella* carcass prevalence and concentration, the following results were found (Table 6): 1) 3 logs reduced the mean risk to zero when homogeneous distribution of cells was applied; 2) 3.75 logs reduced the mean risk to zero when the maximum clustering parameter where applied ( $b=0.1$ ), and 3) 2.5 logs were necessary to reduce the mean risk to zero assuming that the mean concentration of *Salmonella* in the shoulder is reduced after the cutting process.

### 3.3. Sensitivity analysis

According with the regression model (Fig. 3), the most important variables influencing the model were lack of fermentation (B2), short maturation period (12 days), and high concentration of *Salmonella* on the carcass (B). On the contrary, a negative association was observed when 24 days of ripening is applied and or with good fermentation process (G1).

## 4. Discussion

Dry fermented sausage such as Italian-Style salami is very popular in southern Brazil, where it was estimated that a person

**Table 4**

Results of the main variables assessed in the models using five fermentation processes that ranges from optimal (G1, G2, G3) to suboptimal (B1 and B2) and three periods of maturation (12, 15, and 24 days;  $n = 15$  scenarios). Results are given for the worst-case scenario for both carcass prevalence ( $B$ , average of 27.9%) and concentration of *Salmonella* in the carcass ( $B$ , Normal  $(-2.62, 1)$ ) and homogeneous distributions. The indicated means are obtained in 100.000 model iterations, representing 100.000 slaughter days, simulating 20 sausages in each iteration. No. *Salmonella* (100th) represents the maximum number of cfu *Salmonella* found in these 100.000 iterations.

Scenarios	Log reduction, pH, $a_w$	12 days of maturation									
		Mean no. of <i>Salmonella</i> in the batch	Prevalence in the sausages before processing ( $n = 20$ )	Mean no. of <i>Salmonella</i> in the sausages before processing ( $n = 20$ )	Prevalence in the sausages after processing ( $n = 20$ )	Mean no. of <i>Salmonella</i> in the sausages after processing ( $n = 20$ )	No. <i>Salmonella</i> (100th) in a sausage before processing	No. <i>Salmonella</i> (100th) in a sausage after processing	No. <i>Salmonella</i> (100th) in a portion	Mean exposure (cfu)	Mean risk
G1	2.57, 4.9, 0.93	3499,0	34.8%	0.87	0.002%	0.000016	252	1	0	0.000003	$3.2 \times 10^{-10}$
G2	1.51, 5.0, 0.91	3514,5	34.8%	0.88	0.281%	0.00339	156	5	2	0.000264	$2.9 \times 10^{-8}$
G3	0.95, 5.2, 0.90	3515,2	34.7%	0.88	3.82%	0.0465	399	44	5	0.00372	$4.0 \times 10^{-7}$
B1	0.90, 5.3, 0.91	3534,4	34.7%	0.88	6.22%	0.0731	245	31	4	0.00584	$6.3 \times 10^{-7}$
B2	-0.01, 5.8, 0.91	3545,2	34.8%	0.89	34.8%	0.887	365	371	30	0.0709	$7.7 \times 10^{-6}$
Scenarios	Log reduction, pH, $a_w$	15 days of maturation									
		Mean no. of <i>Salmonella</i> in the batch	Prevalence in the sausages before processing ( $n = 20$ )	Mean no. of <i>Salmonella</i> in the sausages before processing ( $n = 20$ )	Prevalence in the sausages after processing ( $n = 20$ )	Mean no. of <i>Salmonella</i> in the sausages after processing ( $n = 20$ )	No. <i>Salmonella</i> (100th) in a sausage before processing	No. <i>Salmonella</i> (100th) in a sausage after processing	No. <i>Salmonella</i> (100th) in a portion	Mean exposure (cfu)	Mean risk
G1	3.3, 4.9, 0.92	3552,9	34.78%	0.89	0.000%	0.00000	283	0	0	0.000000	0
G2	2.2, 5.1, 0.88	3448,1	34.64%	0.86	0.009%	0.00010	295	2	0	0.000008	$8.1 \times 10^{-10}$
G3	1.6, 5.3, 0.87	3488,8	34.66%	0.87	0.151%	0.00176	264	6	2	0.00013	$1.4 \times 10^{-8}$
B1	1.5, 5.3, 0.89	3533,9	34.76%	0.88	0.285%	0.0035	242	8	3	0.00029	$3.1 \times 10^{-8}$
B2	0.4, 5.91, 0.90	3451,3	34.64%	0.86	18.3%	0.306	127	54	6	0.0245	$2.7 \times 10^{-6}$
Scenarios	Log reduction, pH, $a_w$	24 days of maturation									
		Mean no. of <i>Salmonella</i> in the batch	Prevalence in the sausages before processing ( $n = 20$ )	Mean no. of <i>Salmonella</i> in the sausages before processing ( $n = 20$ )	Prevalence in the sausages after processing ( $n = 20$ )	Mean no. of <i>Salmonella</i> in the sausages after processing ( $n = 20$ )	No. <i>Salmonella</i> (100th) in a sausage before processing	No. <i>Salmonella</i> (100th) in a sausage after processing	No. <i>Salmonella</i> (100th) in a portion	Mean exposure (cfu)	Mean risk
G1	5.4, 5.2, 0.90	3488,7	34.66%	0.87	0.000%	0.00000	215	0	0	0.00000	0
G2	4.5, 5.2, 0.84	3501,5	34.73%	0.87	0.000%	0.00000	360	0	0	0.00000	0
G3	3.7, 5.5, 0.80	3471,6	34.73%	0.87	0.000%	0.00000	259	0	0	0.00000	0
B1	3.5, 5.42, 0.84	3524,3	34.74%	0.88	0.000%	0.00000	269	0	0	0.00000	0
B2	1.97, 6.0, 0.84	3489,3	34.69%	0.87	0.032%	0.00036	328	4	1	0.00003	$3.4 \times 10^{-9}$

**Table 5**

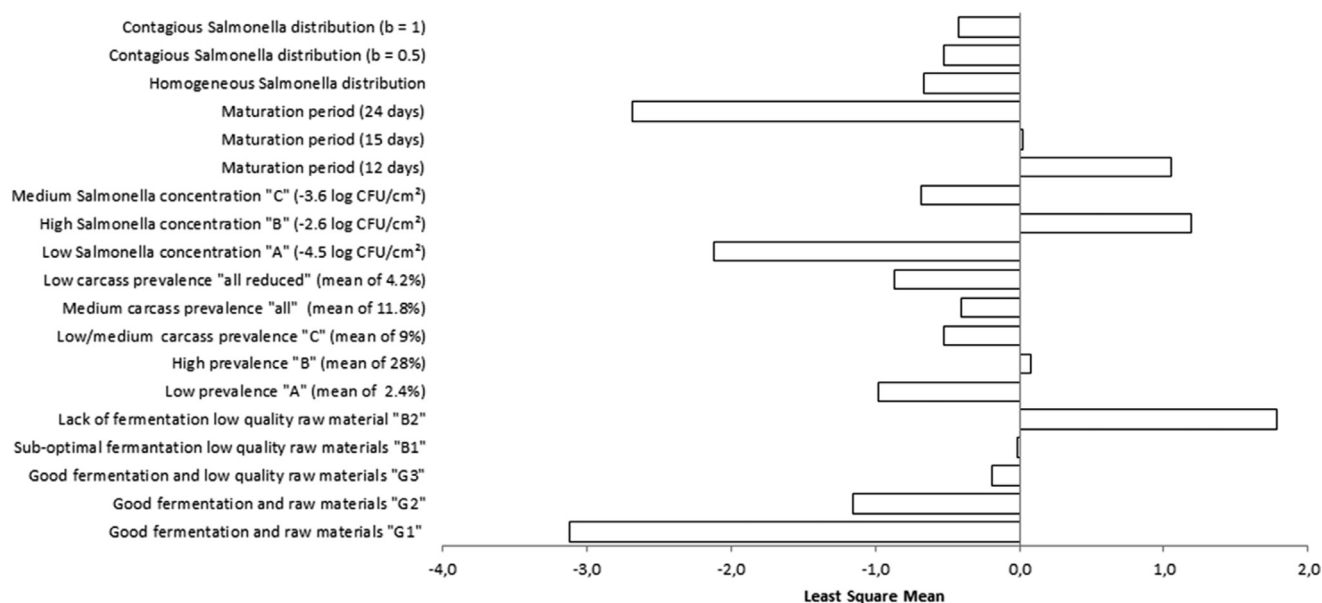
Results of the scenarios used to test the uncertainty around the distribution of *Salmonella* cells in the chopped pork meat. A 'perfect' homogeneity (random) to heterogeneous (clustered cells) were assessed varying the 'clustering' parameter ( $b > 0$ ) of the betabinomial distribution (see Eq. 3). Good fermentation (G3), 15 days of maturation and high prevalence ( $B$ ) and concentration of *Salmonella* in carcasses ( $B$ ) were applied in these scenarios.

'Clustering' parameter ( $b$ )	Prevalence of <i>Salmonella</i> in dry sausage		Mean cfu	Mean risk	Cases
	Before maturation	After maturation			
0.1	12.7%	0.79%	0.011	$9.6 \times 10^{-8}$	13
0.25	19.2%	0.55%	0.007	$6.2 \times 10^{-8}$	8
0.5	24.2%	0.38%	0.005	$3.9 \times 10^{-8}$	5
0.75	26.7%	0.30%	0.004	$2.9 \times 10^{-8}$	4
1	28.2%	0.27%	0.004	$3.6 \times 10^{-8}$	5
10	33.9%	0.15%	0.002	$1.6 \times 10^{-8}$	2
homogeneous	34.8%	0.15%	0.002	$1.5 \times 10^{-8}$	2

**Table 6**

Additional scenarios applied to explore the log reduction required to decrease the risk to during the maturation process in three situations, both tested with the highest parameter of prevalence of *Salmonella* in pork carcasses (average of 27.9%).

Log reduction	High <i>Salmonella</i> concentration and homogeneous distribution		Scenarios High <i>Salmonella</i> concentration and heterogeneous distribution		Reduced <i>Salmonella</i> concentration	
	Mean risk	No. cases	Mean risk	No. cases	Mean risk	No. cases
0.5	$2.2 \times 10^{-6}$	299.22	$2.4 \times 10^{-6}$	321.69	$1.1 \times 10^{-6}$	148.98
1	$3.9 \times 10^{-7}$	52.47	$6.9 \times 10^{-7}$	93.73	$1.3 \times 10^{-7}$	18.16
1.5	$2.9 \times 10^{-8}$	3.95	$1.5 \times 10^{-7}$	20.93	$7.9 \times 10^{-9}$	1.07
2	$3.3 \times 10^{-9}$	0.45	$2.3 \times 10^{-8}$	3.10	$5.4 \times 10^{-10}$	0.07
2.5	$4.9 \times 10^{-10}$	0.07	$2.4 \times 10^{-9}$	0.32	0	0.00
3	0	0.00	$2.7 \times 10^{-10}$	0.04	–	–
3.25	–	–	$1.6 \times 10^{-10}$	0.02	–	–
3.5	–	–	$5.4 \times 10^{-11}$	0.01	–	–
3.75	–	–	0	0.00	–	–



**Fig. 3.** Sensitivity analysis used to identify the most influential variable in the model. A regression model was made using as response variable (Y) the log relative risk and the scenario with good fermentation process (G1), 12 days of maturation, heterogeneous ( $b = 0.5$ ) distribution of *Salmonella* cells in the batch and both high prevalence (B) and concentration of *Salmonella* in the carcasses (B) was used as reference. The explanatory variables (X) were: prevalence (A, B, C, "all", and "all reduced"), *Salmonella* concentration (A, B, C) fermentation scenarios (G1, G2, G3, B1, B2), cells distributions (homogeneous, heterogeneous), and days of maturation (12, 15, 24). The model output is the Least Square Mean of the effects (explanatory variables).

eats on average 398 g/year (IBGE, 2010). Since there is no clear regulation about safety of the process in the region, the only regulation that exists dealing with technical specification, this ready-to-eat product can pose risk to the population. To support the decision making process, it is important to perform a QMRA. Especially in developing countries, where few analyses have been performed (Pouillot et al., 2012), performance of a QMRA may additionally raise the awareness among the authorities about the risks of a product that has a complex manufacturing process and high variation (Kanninen and Puolanne, 2007).

The risks estimated in most of the scenarios analyzed in this study were generally low or zero, especially after 24 days of maturation (giving  $\geq 2$  log reduction in *Salmonella* concentrations), or in other scenarios where the maturation reduced the *Salmonella* concentration with more than 2.2 logs of *Salmonella*, independently of the prevalence and concentration of *Salmonella* in the carcass. An important reason for this result is that *Salmonella* concentrations were low in more than half of the scenarios assessed, resulting in low number of the pathogen in the carcass. As an effect of the carcass cutting, the prevalence of contaminated shoulder was lower

than the prevalence of the carcasses, as expected in a partitioning process (Nauta, 2008). Moreover, the stuffing process reduces the number of *Salmonella* in the sausage units, and at the end of the process, the exposure to the pathogen via a portion of salami was very low in most of the scenarios tested. Swart et al. (2016) reported very low prevalence levels of contaminated portions of fermented sausages at consumption considering only successful fermentation process. Similarly, our model predict very low to zero contamination if the successful fermentation process is applied.

Conversely, higher exposures to *Salmonella* were found mainly in the scenarios that assessed failure or suboptimal fermentation process at 12 or 15 days of maturation times with high prevalence of contaminated carcass and *Salmonella* concentration. In fact, fermentation and drying processes are critical steps for microbiological safety and quality of the product: considerable growth of *Salmonella* was observed during the first 72 h of fermentation. It is desirable that the fermentation reduces the pH to 5.3 or below within the first few hours (Hutkins, 2006), and a failure in this step can result in survival or even growth of *Salmonella*. Nonetheless, a decrease in the number of *Salmonella* in the subsequent

drying process was observed in all the scenarios, including the one that mimicked failure in fermentation. This decrease reduces the risk significantly. Water activity values decreased gradually along the maturation process in all the scenarios assessed, decreasing the probability of survival or growth of *Salmonella*. Koutsoumanis et al. (2004) reported no growth of *Salmonella* in a pH lower than 5.5 and  $a_w$  equal to 0.95 at 15 °C; in the model, after the maturation process was completed, the  $a_w$  and the pH values used ranged from 0.80 to 0.90 and 5.2 to 6.0, respectively. Therefore, these combinations of values used in the models acted as antimicrobial barriers and resulted in a decrease of *Salmonella* over the time. Birk et al. (2016) reported growth of *Salmonella* to a high level during fermentation of sausages without starter culture at 24–25 °C with subsequent reduction by 0.3 to 2.4 logs during the drying process, corroborating with the results found here.

Holzapfel (2002) reports the importance of applying starter cultures since there is a high risk for failure in spontaneous fermentation process. In South of Brazil there is a great variety of salami produced by small manufacturers, in which the production is based more on skill and experience than scientific and technological knowledge (Dalla Santa et al., 2012). These authors reported a high variation in the native microflora in sausages produced by spontaneous fermentation and they pointed out that many sausages analyzed were sold soon after production, after an inadequate maturation time (Dalla Santa et al., 2012). Degenhardt and Sant'anna (2007a) analyzed 20 samples of salami from small scale producer from southern Brazil, and 55% of them had  $a_w$  values greater than 0.94, and one sample had an  $a_w$  value of 0.98. Considering the risks assessed when 12 days of process combined with high concentration of *Salmonella* was used, and the practice of selling the product before maturation, these scenarios mimic a possible situation that can take place in the region, representing a threat for the population. In this study, the maturation period and the failure in the fermentation process along with the level of contamination were important variables correlated with the risks assessed, and therefore local authorities should be aware of the associated health risks. In a study made in the region, *Salmonella* was recovered in 4.4% (4/90) of the salamis sold in street market (Peter et al., 2012). Outbreaks linked to consumption of salami have been reported by the surveillance in the region. From 2000 to 2015, 24 outbreaks out of 2768 reported by the public health authorities were linked to consumption of salami of which *Salmonella* were isolated in eight (Figueiredo – CEVS, personal communication).

In one scenario that exemplified good fermentation, the pH reduced to 5.3 after 24 h, and *Salmonella* growth was observed. Initial parameters used for the pH (6.0) and  $a_w$  (0.99) of the pork meat were extremely high in this scenario. During the initial period evaluated (i.e., the first 72 h), these combination of pH and  $a_w$  values are suitable for *Salmonella* growth. Lachowicz et al. (2012) described that the high pH of meat is related with a larger buffering capacity, suggesting that an extended period of time is needed until pH can drop below the critical level, and sometimes the final pH just will not go down sufficiently. Kanninen and Puolanne (2007) pointed out that the pH values of the meat from exhausted animals remain high (around 6.0 or higher), and that can be unsafe from the microbiological point of view. This reinforces the importance of high quality raw material used to produce dry-fermented sausage, since the model showed growth of *Salmonella* when the chopped pork meat used has high pH and  $a_w$ .

Transmission of the pathogens through the manufacture process of salami is complex and it is reasonable to assume that heterogeneous distribution of *Salmonella* cells can occur due to incomplete mixing of contaminated chopped pork meat. Our results show that the mean risks were higher in scenarios with a more heterogeneous distribution of the cells, which means that

larger log reductions are needed to minimize the risk. This effect of heterogeneity of cells is a consequence of the fact that when cells are more clustered in the minced ingredients, some sausages will contain more *Salmonella* cells after stuffing. This fact reduces the prevalence before maturation. However, after maturation, concentrations will be very low or zero in low contaminated sausages, but it may still be considerable in highly contaminated ones. Apparently, this may lead to an increase of prevalence after maturation (Table 4). Therefore, a higher log reduction will be necessary to inactivate *Salmonella* as compared to a scenario that assumes a homogeneous distribution. This finding demonstrates the importance of including the option of a heterogeneous distribution of cells in the risk assessment.

A limitation of the model is that cross-contamination during the carcass cutting is not considered. It was assumed that during the process of cutting meat from the shoulder, all the *Salmonella* cells will end up in the batch containing minced meat used to produce salami in a day of production. Therefore, it was assumed that all the *Salmonella* cells present in the skin were transferred to the meat. Although this may not be realistic, in fact, in highly contaminated pork carcasses used to produce salami, there is a high probability that most of the contamination present in the skin will contaminate the processing environment (i.e. the cutting room and knife) and end up in the minced meat through cross contamination. Studies made in the region reported high level of contamination of minced pork meat used to produce fresh pork sausage (Castagna et al., 2004), suggesting that in regions where the prevalence of *Salmonella* in the pig population is high, there is a high probability of contamination of the raw material when a batch of contaminated pigs is slaughtered with poor hygienic procedures. As this kind of data is scarce and hard to model, we tested a scenario in which a reduced amount of *Salmonella* from the skin finished in the chopped pork meat employed to produce salami. Using this scenario and high prevalence and *Salmonella* concentration on the pork carcass, the mean risk was reduced to zero after applying 2.5 logs during the inactivation process. In contrast, 3.0 and 3.75 logs were necessary to reduce the risk to negligible levels when homogeneous and heterogeneous scenarios were used, respectively. Using the principle of precaution, particularly in regions where pigs have high exposure to *Salmonella*, higher levels of log inactivation should be recommended.

Uncertainties attending the model were explored using scenarios with distinct parameter values for *Salmonella* concentration, per day variation of carcass prevalence and *Salmonella* cell distribution. Also, what-if scenarios were assessed to explore what happens if fermentation process is suboptimum or fails, or if the product is sold before maturation. However, other sources of variation, not included in the model, might also have some impact on the risk, and need further evaluation when more information is available. As expected, higher risks were found with larger serving sizes. Though, we did not include variation in serving sizes in each simulation because the main objective was to check the impact of the salami manufacturing variables, which are the ones that can be managed, and the distribution of *Salmonella* cells on the risk. Another source of variation not accounted for is the biological variability between bacterial cells that can affect the predicted inactivation, as reported by Aspidou and Koutsoumanis (2015). Furthermore, population heterogeneity of the adaptive stress response (Den Besten et al., 2007) could also affect bacterial resistance during the process of production of salami and might impact the risk estimate.

Nonetheless, if a good process of salami production is applied, such as good selection of raw material, adding starter culture and good control of the fermentation and drying process, and avoiding selling the product before maturation, the resulting log inactivation, at least with the parameters used in this QMRA, is enough to produce a safe product with very low probability of contamination.



## 5. Conclusion

Although in general the mean risks found here were low, selling dry fermented sausage before complete maturation of the product and failure in fermentation can pose a risk to the consumers from the studied region. In all uncertainty and what-if scenarios tested, a maturation period of 24 days can be considered safe, even in a situation with high initial levels of contamination.

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